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Trace element bioaccumulation in grey seals *Halichoerus grypus* from the Faroe Islands

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ABSTRACT : 68 grey seals (*Halichoerus grypus*) were sampled in the Faroe Islands archipelago during the summer period in 1993 to 1995. Concentrations of Cd, Cu, Hg, Se and Zn were measured in liver, kidney and muscle. All elements except Zn exhibited lowest concentrations in muscle. Liver displayed the highest concentrations of Cu, Hg, Se and Zn while kidney exhibited the highest Cd concentrations. However, trace element concentrations within tissues were influenced by sex and age. Thus, females showed clearly higher Cd concentrations than males. Age was the most important factor influencing the concentration of Cd, Hg and Se in liver, and of Cd and Hg in kidney. A strong positive correlation between Cd, Hg and Zn in kidney suggests the occurrence of a detoxification process involving metallothionein proteins. Similarly, strong positive correlation between Hg and Se and molecular Hg:Se ratio close to 1 in liver suggest the occurrence of the demethylation process leading to the formation of mercuric selenide granules. High Hg concentrations might be related to fish consumption by grey seals. However, such a piscivorous diet cannot be

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responsible for the very high Cd concentrations which suggest that the seals diet evolves during the year, and may include a significant proportion of cephalopods in other seasons. Finally, in comparison to other grey seal populations, very high Cd concentrations in grey seal tissues also suggest that Faroe Islands are submitted to sub-arctic influences.

Key words : Heavy metals ; Distribution ; Detoxification ; Marine mammals ; Pinnipeds ; Sub-arctic

INTRODUCTION

Situated in the Northeast Atlantic Ocean, the Faroe Islands are a relatively long distance from major sources of anthropogenic pollution. Nevertheless, marine top predators from this area, such as long finned pilot whales *Globicephala melas* or white-sided dolphins *Lagenorhyncus acutus*, exhibited very high cadmium (Cd) and mercury (Hg) concentrations in their tissues (Julshamn et al. 1987, Caurant et al. 1994, Dam 2001, Gallien et al. 2001). These concentrations were much higher than those reported for individuals from temperate areas which are subject to significant pollution from human activities (Mackey et al. 1995, Law et al. 2001). In fact, toxic metals in top marine predators from the Faroe Islands often reach the threshold level at which metabolic disorders appear in humans (Nogawa 1984). However, such effects are difficult to detect in wild animals such as marine mammals. Recently, Gallien et al. (2001) investigated cellular alterations in relation to Cd concentrations in the kidney of white-sided dolphins from the Faroe Islands. This study reported the occurrence of calcium phosphate granules in this organ which could result from an effective detoxification process of Cd but could also lead to some alteration of the functioning of the kidney. Cd could also be detoxified by proteins regulating essential elements (e.g. copper, Cu, or zinc, Zn) such as ferritin or metallothionein (Das et al. 2000). Considering Hg, other processes are involved in its co-precipitation with selenium (Se) to produce non-toxic mineral granules of tiemannite (Martoja & Berry 1980, Nigro & Leonzio 1996).

Top marine predators are mainly exposed to trace elements through the food (Aguilar et al. 1999). Thus, the consequence of a specialised diet for a marine mammal is an exposure either to very low or very large amounts of poorly or highly concentrated toxic element in the prey. For example, very high Cd concentrations in the kidney of pilot whales from the Faroe Islands have been related to their teutophageous diet (Caurant & Amiard-triquet 1995, Bustamante et al. 1998a). However, the lactating pilot whales displayed higher Hg concentrations which have been explained by an increase of the fish proportion in their diet during the nursing period because fish are more energetic than cephalopods (Caurant et al. 1994). Following these conclusions, piscivorous marine mammals such as seals would be expected to have high Hg and low Cd concentrations in their tissues.

In this study, the concentration and distribution of five trace elements, Cd, Cu, Hg, Se and Zn, have been determined in a reported piscivorous marine mammal species (Mikkelsen et al. 2002) from the Faroe Islands, the grey seal *Halichoerus grypus*. Therefore, the primary objectives of this work were to investigate the influence of biological (sex, maturity stage and age), ecological (diet) and geographical factors on the bioaccumulation of these trace elements. Particular attention was granted to interactions between toxic Cd and Hg with essential Cu, Se and Zn. Furthermore, trace element concentrations were compared to other seal species and to grey seals from other areas. Cd and Hg concentrations were also considered from an ecological point of view.

MATERIALS AND METHODS

Sampling

During the summer of 1993 to 1995, 68 grey seals were caught at the Faroe Islands (Fig. 1), in an co-operation between the Museum of Natural History of the Faroe Islands and the Norwegian Institute of Fisheries and Aquaculture, with the aim to study the diet of this poorly investigated population (Mikkelsen et al. 2002). These sampled animals have given the unique opportunity to collect organs and tissues for metallic and organic contaminant investigations (Dam 2001). Samples for trace element analysis, i.e. liver, kidney and muscle, have been stored at –20°C in individual plastic bags until use. Furthermore, teeth were used for age determination. Biological parameters of the seals (length, weight, blubber thickness, and age) for each sex and maturity stage are given in Table 1.

Analytical procedure

All the material used for the treatment of the samples was cleaned, then decontaminated during 24 hours in a solution composed by 35 ml of HNO_3 65% and 50 ml of HCl 36% for 1 L Milli-Ro quality water.

Fresh samples were freeze dried and then grounded to powder. The mean of the ratio between wet weight and dry weight was about 3.4 for liver and muscle, and 4.1 for kidney. Two aliquots of approx. 200 mg of each homogenised dry sample were digested with 3.5 ml of 65% HNO_3 at 60°C for 3 d. The digested contents were then diluted to 10 ml in Milli-Q quality water. Cd, Cu, Se and Zn were assayed using flame and graphite furnace atomic absorption spectrophotometer Varian 250 Plus with deuterium background correction.

For Hg measurements, aliquots ranging from 0.5 to 10 mg of dried material were analysed directly for total Hg in an Advanced Mercury Analyser spectrophotometer, Altec AMA 254. Hg determination involved evaporation of Hg by progressive heating until 800°C under oxygen atmosphere for 3 min and subsequent amalgamation on a Au-net. Afterwards, the net was heated to liberate the collected mercury and was subsequently measured by UV atomic absorption spectrophotometry.

The analytical accuracy was assessed using dogfish liver DOLT-2 (NRCC) and dogfish muscle DORM-2 (NRCC) as reference materials. Analyses were also validated through international intercalibration exercises (e.g. Coquery et al. 2001). The standards were treated and analysed under the same conditions as the seal samples. Results were in good agreement with the certified values and recoveries of the metals ranged from 93 to 105%. Detection limits (µg.g^{-1} dry wt) were 0.004 for Cd, 0.5 for Cu, 3 for Zn, 0.8 for Se, and 0.005 for total Hg. All trace element concentrations in seal tissues are reported in µg.g^{-1} wet wt (wwt).

Statistical analysis

Comparison of metal concentrations between species were tested by one-way ANOVA (after log transformation of data when necessary) followed by the Tukey multiple comparison test in the MINITAB 13.1 Software. Hypothesis of normal distribution was tested using the Anderson-Darling test and equality of variance by the Bartlett test. The significance level for statistical analyses was always set at $\alpha = 0.05$.

RESULTS

Levels of trace elements and organotropism

Cd, Cu, Hg, Se and Zn concentrations in liver, kidney and muscle for each sex and maturity stage are presented in Table 2. Cd and Hg concentrations varied greatly among tissues. Kidney exhibited the highest Cd concentrations (15.8 ± 24.6 µg.g^{-1} wwt) followed by liver (5.06 ± 8.63 µg.g^{-1} wwt) while muscle showed Cd concentrations several order of magnitude lower (0.05 ± 0.14 µg.g^{-1} wwt) (Table 2). As expected for a toxic element, the coefficients of variation for Cd were particularly elevated in all the tissues, i.e. 155% for kidney, 170% for liver and 280% for muscle. Among the three tissues analysed, liver exhibited the highest Hg concentrations (59.7 ± 70.4 µg.g^{-1} wwt) followed by kidney (2.93 ± 2.47 µg.g^{-1} wwt) and muscle (0.74 ± 0.65 µg.g^{-1} wwt) (Table 2). The coefficients of variation for Hg were relatively elevated, particularly for liver (118%) but also for kidney and muscle (84 and 88%, respectively). Liver also exhibited the highest Se concentrations (23.0 ± 26.2 µg.g^{-1} wwt) followed by kidney (5.47 ± 1.43 µg.g^{-1} wwt) and muscle (0.27 ± 0.10 µg.g^{-1} wwt). For Se, the coefficients of variation were lower than 40% in kidney and muscle but higher than 100% in liver.

Liver exhibited the highest Cu concentrations (38.7 ± 18.1 $\mu g.g^{-1}$ wwt) while concentrations were relatively low in kidney and muscle (3.1 ± 0.5 $\mu g.g^{-1}$ wwt and 1.5 ± 0.3 $\mu g.g^{-1}$ wwt, respectively). As expected for an essential element, coefficients of variation were low for Cu, i.e. 15%, 25% and 47% in kidney, muscle and liver, respectively. Liver also showed the highest Zn concentrations (57 ± 10 $\mu g.g^{-1}$ wwt) followed by muscle (41 ± 9 $\mu g.g^{-1}$ wwt) and kidney (27 ± 7 $\mu g.g^{-1}$ wwt). For Zn, the coefficients of variation were lower than 30% in the three tissues analysed.

Influence of gender

In the grey seals from the Faroe Islands, Cd was the only element exhibiting a significant difference between sexes. Thus, Cd concentrations in kidney were significantly higher ($p < 0.001$) in adult females than in adult males (i.e. 40.8 ± 28.8 and 10.5 ± 4.69 $\mu g.g^{-1}$ wwt, respectively) resulting from Cd accumulation rates 5 times higher in females (Fig. 2). In liver, males exhibited significantly lower Cd concentrations ($p < 0.001$) with 1.76 ± 0.27 $\mu g\ Cd.g^{-1}$ wwt while females exhibited mean concentrations of 13.2 ± 11.0 $\mu g.g^{-1}$ wwt, resulting from Cd accumulation rates 10 times higher (Fig. 2).

No other significant differences between sexes were found for the other metals in grey seals tissues.

Influence of maturity stage and age

Cd concentrations in the three tissues of mature individuals are higher than those of immature animals ($F = 47.40$ $p < 0.001$), (Table 2). Moreover, Cd was significantly correlated with age in kidney and liver (Fig. 2). In the former organ, the correlation is linear for both male and female ($r = 0.947$ $p < 0.001$ and $r = 0.818$ $p < 0.001$, respectively). However, the correlation between hepatic Cd and age fitted a logarithmic adjustment for males ($r = 0.747$ $p < 0.001$) but a linear one for females ($r = 0.672$ $p < 0.001$) (Fig. 2).

Higher Hg concentrations in the three tissues of mature individuals are encountered compared to immature ($F = 23.91$ $p < 0.001$). Hepatic and renal Hg concentrations increased linearly with age ($r = 0.882$ $p < 0.001$ and $r = 0.733$ $p < 0.001$, respectively) (Fig. 3).

Hepatic Se concentrations differed significantly ($F = 82.48$ $p < 0.001$) between immature (5.69 ± 6.51 $\mu g.g^{-1}$ wwt) and mature individuals of both sexes (49.2 ± 22.9 $\mu g.g^{-1}$ wwt). Se concentrations were correlated with age only in liver ($r = 0.857$ $p < 0.001$). Furthermore, hepatic Cu concentrations were significantly lower ($F = 6.86$ $p < 0.002$) in immature (32.3 ± 15.9 $\mu g.g^{-1}$ wwt) than in mature seals (48.6 ± 16.9 $\mu g.g^{-1}$ wwt) and renal Cu concentrations in both sexes were significantly higher ($F = 6.02$ $p < 0.004$) in immature seals (3.2 ± 0.4 $\mu g.g^{-1}$ wwt) than in the mature ones (2.9 ± 0.5 $\mu g.g^{-1}$ wwt). However, Cu concentrations in the tissues of grey seals were not correlated with age.

In both sexes, mature seals showed significantly higher Zn concentrations in kidney ($F = 55.36$ $p < 0.001$) than did the immature individuals (34.2 ± 6.6 $\mu g.g^{-1}$ wwt and 22.8 ± 2.5 $\mu g.g^{-1}$ wwt, respectively). Therefore, renal Zn increased linearly with age ($r = 0.796$ $p < 0.001$). However, Zn concentrations in liver and muscle have not been found to be correlated with age.

Metallic correlation

Table 3 shows the correlation between trace elements within the three tissues. In kidney, Zn was correlated with Cd ($r = 0.907$ $p < 0.001$) and Hg ($r = 0.698$ $p < 0.001$) (Fig. 4). Renal Hg concentrations were also strongly correlated to Se ($r = 0.688$ $p < 0.001$) and to Cd ($r = 0.773$ $p < 0.001$) (Fig. 4).

In liver, Cd concentrations were correlated to Se, Hg and Zn ($p < 0.001$, Table 3). Hg and Se concentrations exhibited a linear relationship for both sexes ($r = 0.989$ $p < 0.001$) (Fig. 5). Thus, the calculated molar ratio between Hg and Se was also found to be correlated with age ($r = 0.642$ $p < 0.001$) and tended to a Hg:Se ratio close to 1 (Fig. 5).

In muscle, only a negative correlation was found between Cu and Hg (Table 3).

DISCUSSION

In contrast to traditionally hunted pinniped species, i.e. harp and hooded seals, which have been extensively studied for trace elements and organic compounds bioaccumulation (see the review of Muir et al. 1992), grey seal has only been investigated in few cases and often with few individuals (Morris et al. 1989, Frank et al. 1992, Law et al. 1992, 1998, Teigen et al. 1999, Dam 2001, Nyman et al. 2002), probably because most of the grey seal populations are either protected in their living areas or not traditionally hunted. Generally, only stranded animals, by-caught during fisheries or killed in small numbers with special authorisations are used for ecotoxicological analyses. Thus, the sampling realised in the Faroe Islands to study the grey seal diet in this archipelago provided a great opportunity to investigate the bioaccumulation of trace elements in a supposed healthy marine mammal population.

As in other pinniped species (e.g. Wagemann et al. 1988, Dietz et al. 1996, de Moreno et al. 1997, Julshamn & Grahl-Nielsen 2000, Fant et al. 2001), each trace element analysed was mainly concentrated in a specific tissue. Thus, the highest concentrations of Cu, Hg, Se and Zn were found in the liver whereas kidney contained the highest Cd concentrations (Table 2). However, within a given population, several factors like sex, reproductive status, age and diet greatly influence the concentration of trace elements within tissues.

In marine mammals, trace elements are mainly incorporated in the body via food, thus diet can be considered as the main factor that determines the load of a species (Aguilar et al. 1999). Therefore, intake via food is supposed to represent the bulk of trace element intake. Diet has been investigated on the same individuals of this study, and showed that grey seals from the Faroe Islands feed exclusively on fish during the summer period; males and females displaying a similar diet according to their stomach contents (Mikkelsen et al. 2002). However, very elevated Cd concentrations in their tissues is not in accordance with a piscivorous diet. Indeed, very low Cd exposure would result from fish consumption as most fish species generally display relatively low Cd concentrations when considering the whole animal (Bustamante et al. 2003). In the light of the present results, it seems likely that the diet of grey seals in Faroese waters changes during the year, and thus include a significant proportion of cephalopods, strongly increasing their exposure to Cd (Bustamante et al. 1998a). In fact, the diet of grey seals from the northern temperate waters appears to be very dependent of the season (Hammond et al. 1994, Pierce et al. 1990, 1991). Reported results were closely similar to those of Mikkelsen et al (2002) for the summer period, but in autumn, winter and spring, grey seals increased the proportion of cephalopod in their diet. Such a shift in diet might occur for the Faroe grey seals, and would explain the high Cd concentrations found in kidney and liver.

Even if investigations on the grey seals diet in the Faroe Islands showed that there is no sex-related difference in their diet (Mikkelsen et al. 2002), Cd concentrations determined in liver and kidney revealed a significant gender-related difference. Thus, females exhibited significantly higher renal and hepatic concentrations than males (Table 2, Fig.1). Nyman et al. (2002) also reported higher Cd concentrations in the liver and kidney of female grey seals from Baltic Sea and from Sable Island. Similar gender difference observed in the Antarctic fur seal (*Arctocephallus gazella*) and the Baïkal seal (*Phoca sibirica*) was partly explained by different feeding habits between males and females (de Moreno et al. 1997, Watanabe et al. 1998). Such a difference in metal concentrations is sometimes attributed to the sexual dimorphism (Caurant et al. 1994, Watanabe et al. 1998). This could be the case here since males weigh up to 290 kg while females do not exceed 191 kg. Moreover, female seals seem to have higher feeding rates than males, essentially because of the energetic cost of reproduction , viz. gestation and lactation, while males reduce drastically their feeding during the mating and moulting seasons. For example, an adult ringed seal weighing about 70 kg might ingest 1200 to 1300 kg of food per year, whereas an adult female weighing about 60 kg needs 1600 kg per year (Ryg & Øritsland 1991). Considering that males and females feed on the same species, and that grey seals might have a similar feeding dimorphism as the ringed seals, the uptake of Cd would be greater for females because of the greater quantities of ingested food *vs* body weight. These results also suggest that Cd transfer to foetus through the placenta or to calves via milk is very limited. It cannot be excluded that this variation could be associated with a difference in metabolic pathways linked to hormone cycle, obviously different in both sexes (Caurant et al. 1994). Increase of trace element concentrations with age was observed for Cd in kidney and liver but gender differences in the accumulation rates are noteworthy (Fig. 2). In liver, increasing of Cd concentrations with age was different between sexes, the accumulation fitted a logarithmic adjustment only for males. Such a logarithmic shape for Cd accumulation has been previously shown in the liver of the long-finned pilot whale from the Faroe Islands (Caurant et al. 1994) This may suggest an equilibrium between assimilation and excretion of Cd in liver as shown for humans after 50 years old (Friberg et al. 1974). In grey seals, this equilibrium would appear from 12 years old in males while in females, Cd was linearly accumulated with age. However, the present sampling lacks very old females as their maximum age was 27 years old while they can live more than 35 years.

When comparing trace element concentrations between seal species, Zn and Cu concentrations appear to be in the same order of magnitude than those reported for other pinniped species (Table 4), probably because these essential elements are submitted to homeostatic regulation in marine mammal tissues (Law 1996). However, Cd, Hg and Se exhibited among the highest values reported for pinnipeds in the literature. Thus, hepatic Se in the grey seal from the Faroe Islands varied from 1.17 to 99.9 µg.g^{-1} wwt and were comparable to the high hepatic Se concentrations ranging from 1.30 to 65.3 µg.g^{-1} wwt in the ringed seal from Western Canadian Arctic (Wagemann et al. 1996). High Se concentrations in liver were also reported for grey seals from the Baltic sea (2.7-79.2 µg.g^{-1} wwt) and from Sable Island (9.3-83 µg.g^{-1} wwt) (Nyman et al. 2002). Concentrations of Hg and Cd are globally well documented in seals (Table 4). Generally, seals from polar areas exhibited high levels of hepatic Hg and renal Cd. Surprisingly for a species largely distributed in the temperate waters, grey seals also generally displayed very high Hg concentrations in liver. Present results for grey seals from the Faroe Islands follow this trend as their Hg concentrations vary from 1.13 to 238 µg.g^{-1} wwt in liver. Concerning Cd, grey seals from the Faroe Islands appeared to be atypical compared to those from Baltic Sea or Sable Island (Table 4). Indeed, they displayed Cd concentrations in kidney comparable to those of seals from the Arctic and clearly higher Cd concentrations than grey seals from Baltic Sea or Sable Island.

In summary, grey seals from the Faroe Islands exhibited among the highest renal Cd and hepatic Hg concentrations compared to other pinnipeds, particularly those from lower latitudes (Table 4). Very high Hg concentrations in grey seals from the Irish Sea were related to feeding habits in a very contaminated area, the Liverpool Bay, where their preys are enriched in Hg (Law et al. 1992). In the Faroe Islands, such an enrichment due to industrial contamination does not exist. Consequently, the high Hg concentrations in the tissues of the grey seals might be considered as the consequence of background levels. In contrast, global

Cd enrichment in the Arctic remained unclear. In fact, local enrichment have been noted when comparing marine mammal populations throughout the Arctic but it does not explain this phenomenon on a global scale (Wagemann et al. 1996). Several authors have proposed that a phenomenon of Cd abnormality occurs in polar and subpolar areas, leading to very high Cd concentrations in many invertebrates and vertebrates species (Petri & Zauke 1993, Bargagli et al. 1996, Bustamante et al. 1998b, Zauke et al. 1999). Several results suggest that such an abnormality has also to be considered in the Faroe Islands. Indeed, similarly to polar regions, soluble trace element concentrations in seawater appear to be very low around this area (Mart & Nürnberg 1984), but high Cd concentrations, reaching up 9.06 ± 3.38 $\mu g.g^{-1}$ wwt and 6.55 ± 1.97 $\mu g.g^{-1}$ wwt have been found in different cephalopod species (i.e. *Loligo forbesi*, *Todarodes sagittatus* and *Eledone cirrhosa*) and in the queen scallops *Aequipecten opercularis* caught around the archipelago, respectively (Bustamante 1998, Bustamante et al. 1998a). Similarly, in coastal waters, the dog-whelks *Nucella lapillus* and limpets *Patella* sp. displayed Cd concentrations of 28 $\mu g.g^{-1}$ wwt and 10 $\mu g.g^{-1}$ wwt, respectively (Dam 2000). Furthermore, other marine mammal species from the Faroe Islands, i.e. the long-finned pilot whales *Globicephala melas* and the white-sided dolphins *Lagenorhynchus acutus*, also exhibited very high concentrations of Cd and Hg in their tissues (Julshamn et al. 1987, Caurant et al. 1994, Gallien et al. 2001; Olsen et al., in press). Marine mammals are currently considered as useful bioindicators of trace element contamination in the environment because of their high trophic level and their wide distribution. Differences in trace element concentrations between individuals from different areas may be due to different exposures through food rather than metabolic differences. In consequence, continued release of toxic metals such as Cd or Hg in the environment would be of particular concern in these areas.

Toxicological aspects for individuals

Within a single individual, the behaviour of trace elements depends on the different regulation and/or detoxification processes, leading to their storage in the tissues or to their excretion. Extremely high levels of toxic Hg and Cd in mature grey seals may trigger cellular and physiological damages of the organs of storage. Thus, renal dysfunction is expected to appear when Cd concentrations exceed 50 $\mu g.g^{-1}$ wwt in humans (Elinder & Järup 1996). Mature grey seals exhibited Cd concentrations in kidney close to that limit (36.3 ± 28.8 $\mu g.g^{-1}$ wwt) with the oldest females exceeding it by far (see Fig 1). Furthermore, most of the mature grey seals from this study exhibited Hg concentrations exceeding the threshold levels of 60 $\mu g.g^{-1}$ for liver damage in mammals (AMAP 1998). Nevertheless, according to their body mass and their blubber thickness (Table 1), seals were found to be in good health. This strongly suggest that grey seals from the Faroe Islands would have developed very efficient detoxification process that allow them to cope with such high Cd and Hg concentrations in their tissues.

Liver and kidney of grey seals have been reported to contained high levels of metallothionein which could trap toxic metals such as Cd (Olafson & Thompson 1974, Teigen et al. 1999). The primary role of metallothionein is to bind essential metals like Cu and Zn and to serve as an intracellular protein involved in metal homeostasis (Vallee 1991). However, Cd shows a great affinity for metallothionein, leading to a competition with Zn for binding sites and to a reduction of the toxic effects of Cd (Tsalev & Zaprianov 1983). Thus, displaced Zn would induce the metallothionein synthesis, increasing the number of binding sites within the renal cells. If this hypothesis is true, Cd and Zn would be accumulated jointly. Significant correlation between Zn and Cd (Fig. 4) in the kidney of grey seals from the Faroe Islands strongly suggest the occurrence of an effective detoxification process of Cd involving metallothionein in this organ.

In grey seal liver, Hg concentrations were strongly positively correlated with Se during the life span (Fig. 5). It has been reported that selenium apparently exerts a protective influence against Hg toxicity and seems to govern its hepatic accumulation by the formation of mercuric selenide (HgSe) granules (Pelletier 1985, Cuvin-Aralar & Furness 1991, Nigro & Leonzio 1996). HgSe granules (tiemannite) have been identified in the liver of seabirds, marine mammals and humans (Martoja & Berry 1980, Pelletier 1985, Hansen et al. 1989, Nigro & Leonzio 1996). Moreover, many studies also reported that Hg and Se are correlated in a 1:1 molar ratio as in HgSe (e.g. Koeman et al. 1973, Nielsen & Dietz 1990, Wagemann et al. 1998). Grey seals from the Faroe Islands exhibited a Hg:Se molar ratio in liver tending towards 1 (Fig. 5). This strongly suggests the presence of tiemannite granules as a result of coprecipitation of Hg with Se. A molar ratio between hepatic Hg and Se close to 1 in grey seals were found to occur for mature males and females. They displayed the same kinetics of accumulation with age which supposes a similar detoxification process of Hg in liver for both sexes. This also suggests that pregnancy and lactation are not efficient processes of elimination for females. Moreover, the annual moult, probably participating to the elimination of some Hg, is not sufficient to counteract the high assimilation of Hg.

CONCLUSION

Grey seals from the Faroe Islands showed very high Cd and Hg concentrations in their tissues. These Cd concentrations are particularly elevated compared to those in other grey seal populations. The Cd and Hg concentrations in the oldest individuals are clearly higher than the threshold levels for toxic effect which induces a threat to their health status. However, very efficient detoxification processes, involving metallothionein protein and co-precipitation of Hg with Se, probably account for the good condition of the analysed seals. Furthermore, the present results highlight that Faroe Islands also show a Cd “abnormality” as previously reported in polar oceans. Finally, the very high tissular Cd contents lead us to suspect important changes in the diet of the seals during the year. Hence, seals may also feed on cephalopods, and that might account for the high Cd concentrations found in the oldest individuals.

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Caption to figures

Figure 1. Map of the Faroe Islands. Number and position of grey seals sampled in the summer period in 1993-95 are indicated.

Figure 2. *Halichoerus grypus*. Renal and hepatic Cd concentrations ($\mu g.g^{-1}$ wwet) in relation to age in male (●) and female (▲)

Figure 3. *Halichoerus grypus*. Renal and hepatic Hg concentrations ($\mu g.g^{-1}$ wwet) in relation to age

Figure 4. *Halichoerus grypus*. Relations between Zn, Cd and Hg and between Se and Hg concentrations ($\mu g.g^{-1}$ wwet) in kidney

Figure 5. *Halichoerus grypus*. Correlation between hepatic Se and hepatic Hg concentrations ($\mu atom.g^{-1}$ dwt) and molar Hg:Se in relation to age

Table 1. *Halichoerus grypus*. Characteristics (sex, length, weight, dorsal blubber thickness and age) of the individuals sampled in the Faroe Islands between 1993 and 1995

Sex Sexual maturity	N	Length (cm)	Weight (kg)	Blubber thickness (mm)	Age (years)
Male					
Mature	4	212.0 ± 11.9	229.3 ± 68.4	35.0 ± 10.0	17.0 ± 5.2
Immature	18	136.5 ± 15.7	56.8 ± 20.8	22.1 ± 4.7	1.9 ± 1.3
Whole	22	150.3 ± 33.3	89.6 ± 76.6	24.9 ± 7.7	4.6 ± 6.4
Female					
Mature	23	176.4 ± 8.5	152.0 ± 20.8	39.7 ± 8.5	14.3 ± 4.5
Immature	23	133.7 ± 10.5	49.4 ± 11.4	22.6 ± 4.0	2.3 ± 1.1
Whole	46	154.6 ± 23.6	99.6 ± 54.4	31.1 ± 11.1	8.3 ± 6.9
Whole individuals	68	153.2 ± 27.0	96.4 ± 61.9	29.1 ± 10.5	7.1 ± 6.9

Table 2. *Halichoerus grypus*. Means ± SD and range of Cd, Cu, Hg, Se and Zn concentrations (µg.g^{-1} wwet) in liver, kidney and muscle. N : number of individuals

Tissues			Cd		Cu		Hg		Se		Zn	
Sex	Maturity	N	Mean ± SD	range	Mean ± SD	range	Mean ± SD	range	Mean ± SD	Range	Mean ± SD	range
Liver												
Male	Mature	4	1.76 ± 0.27	1.50-2.09	31.2 ± 8.1	23.8-41.8	123 ± 70.9	46.4-199	41.2 ± 24.7	18.8-53.1	62 ± 14	50-76
	Immature	18	0.59 ± 0.80	0.06-3.61	29.6 ± 18.2	5.5-79.6	10.3 ± 15.9	1.56-65.4	4.69 ± 5.63	1.43-23.7	53 ± 8	30-63
Female	Mature	23	13.2 ± 11.0	2.29-51.9	51.6 ± 16.3	28.3-85.3	133 ± 58.0	34.2-238	50.6 ± 22.8	13.5-99.9	60 ± 8	46-75
	Immature	23	1.04 ± 1.07	0.15-4.05	34.4 ± 14.0	7.2-61.1	14.0 ± 18.7	1.13-90.1	6.47 ± 7.15	1.17-35.6	56 ± 10	31-75
Both sex		68	5.06 ± 8.63	0.06-51.9	38.7 ± 18.1	5.5-85.3	59.7 ± 70.4	1.13-238	23.0 ± 26.2	1.17-99.9	57 ± 10	30-76
Kidney												
Male	Mature	4	10.5 ± 4.69	6.30-15.3	2.5 ± 0.4	2.0-2.9	7.89 ± 5.86	2.85-15.9	6.49 ± 2.22	4.02-8.95	29 ± 4	23-31
	Immature	18	2.07 ± 1.72	0.39-7.43	3.3 ± 0.5	2.6-4.6	1.77 ± 1.22	0.41-4.53	5.10 ± 1.24	2.72-6.87	23 ± 2	18-28
Female	Mature	23	40.8 ± 28.8	8.91-155	3.0 ± 0.4	2.2-3.9	4.49 ± 1.28	1.63-7.15	5.93 ± 1.31	3.50-9.42	35 ± 6	26-53
	Immature	23	2.52 ± 1.50	0.96-6.23	3.1 ± 0.4	2.4-3.6	1.41 ± 0.75	0.61-4.06	5.10 ± 1.40	2.63-7.15	23 ± 3	19-28
Both sex		68	15.8 ± 24.6	0.39-155	3.1 ± 0.5	2.0-4.6	2.93 ± 2.47	0.41-15.9	5.47 ± 1.43	2.63-9.42	27 ± 7	18-53
Muscle												
Male	Mature	4	0.03 ± 0.02	0.01-0.05	1.1 ± 0.2	1.0-1.2	1.99 ± 1.86	0.59-4.61	0.27 ± 0.07	0.18-0.34	33 ± 9	25-44
	Immature	17	0.01 ± 0.01	<0.01-0.04	1.7 ± 0.3	1.4-2.4	0.53 ± 0.28	0.18-1.21	0.29 ± 0.14	0.08-0.50	42 ± 9	24-57
Female	Mature	23	0.12 ± 0.23	0.01-1.13	1.4 ± 0.3	1.1-1.9	1.00 ± 0.48	0.31-2.63	0.23 ± 0.05	0.14-0.36	44 ± 9	24-53
	Immature	23	0.01 ± 0.01	<0.01-0.06	1.6 ± 0.4	1.2-2.9	0.44 ± 0.21	0.22-1.06	0.29 ± 0.10	0.11-0.51	40 ± 8	24-51
Both sex		67	0.05 ± 0.14	<0.01-1.13	1.5 ± 0.3	1.0-2.9	0.74 ± 0.65	0.18-4.61	0.27 ± 0.10	0.08-0.51	41 ± 9	24-57

Table 3. *Halichoerus grypus*. Correlation between trace elements within the different tissues.
Not underlined : $p<0.05$; underlined : $p<0.001$.

Elements	Kidney	Liver	Muscle
Cd	+ Hg, + Zn	+ Hg, + Se, + Zn	
Cu	- Hg	+ Hg, + Se, + Zn	- Hg
Hg	+ Cd, - Cu, + Se, + Zn	+ Cd, + Cu, + Se	- Cu
Se	+ Hg,	+ Cd, + Cu, + Hg,	
Zn	+ Cd, + Hg	+ Cd, + Cu	

Table 4. Concentrations of hepatic Hg and renal Cd (μg.g^{-1} wwet) in various species of Pinnipeds sampled in different regions and habitats.

Regions	Species	Hepatic Hg			Renal Cd			Source
		N	Mean ± SD	range	N	Mean ± SD	range	
Arctic								
Greenland sea	Harp seal	29	-	0.14-7.7	29	-	0.1-101	Julshamn & Grahl-Nielsen (2000)
Greenland sea	Hooded seal	22	-	0.26-179	22	-	0.13-207	Julshamn & Grahl-Nielsen (2000)
Arctic ocean	Harbour seal	38	-	0.2-66	-	-	-	Frank et al. (1992
West arctic Canada	Ringed seal	145	32.9 ± 35.2	0.2-2.2	144	21.1 ± 14.2	0.12-87.1	Wagemann et al. (1996)
East arctic Canada	Ringed seal	115	8.3 ± 7.0	0.4-38.7	35	47.7 ± 23.3	8.98-111	Wagemann et al. (1996)
Eureka (Canada)	Ringed seal	8	26.5 ± 36.9	0.4-143	17	15.6 ± 13.8	0.009-46.3	Wagemann et al. (1996)
Subarctic								
Faroe Islands	Grey seal	68	59.7 ± 70.4	1.13-237.7	68	15.8 ± 24.6	0.39-154.7	This study
Baltic Sea	Grey seal	20	78 ± 84	15-348	20	3.4 ± 1.7	1.4-7.6	Nyman et al. (2002)
Baltic Sea	Grey seal	19	-	7.03-92	19	-	0.25-2.72	Frank et al. (1992)
Sable Island	Grey seal	20	109 ± 72	27-278	20	1.8 ± 1.1	3.0-20	Nyman et al. (2002)
Norway Oslofjord	Harbour seal	28	4.1	0.1-30	-	-	-	Skaare et al. (1990)
Norway southern coast	Harbour seal	26	16	0.2-89	-	-	-	Skaare et al. (1990)
Norway northern coast	Harbour seal	17	7.9	0.4-43	-	-	-	Skaare et al. (1990)
St Laurent gulf	Harp seal	20	10.4 ± 8.1	1.3-30.1	20	28 ± 9	9.5-46.3	Wagemann et al. (1988)
Baltic Sea	Ringed seal	20	53	6.5-124	20	-	0.8-6	Fant et al. (2001)
Northern temperate waters								
Ireland sea	Grey seal	2	0.07	0.06-0.08	2	2.1	1.7-2.5	Morris et al. (1989)
Ireland sea	Grey seal	21	108 ± 132	0.5-430	-	-	-	Law et al. (1992)
Liverpool Bay	Grey seal	2	595	330-860	-	-	-	Law et al. (1992)
Ireland sea	Harbour seal	13	51 ± 58	1-170	-	-	-	Law et al. (1992)
Lakes								
Baikal lake	Baikal seal	41	2.3 ± 2.6	0.2-9.1	40	1.8 ± 0.8	0.6-3.6	Watanabe et al. (1998)

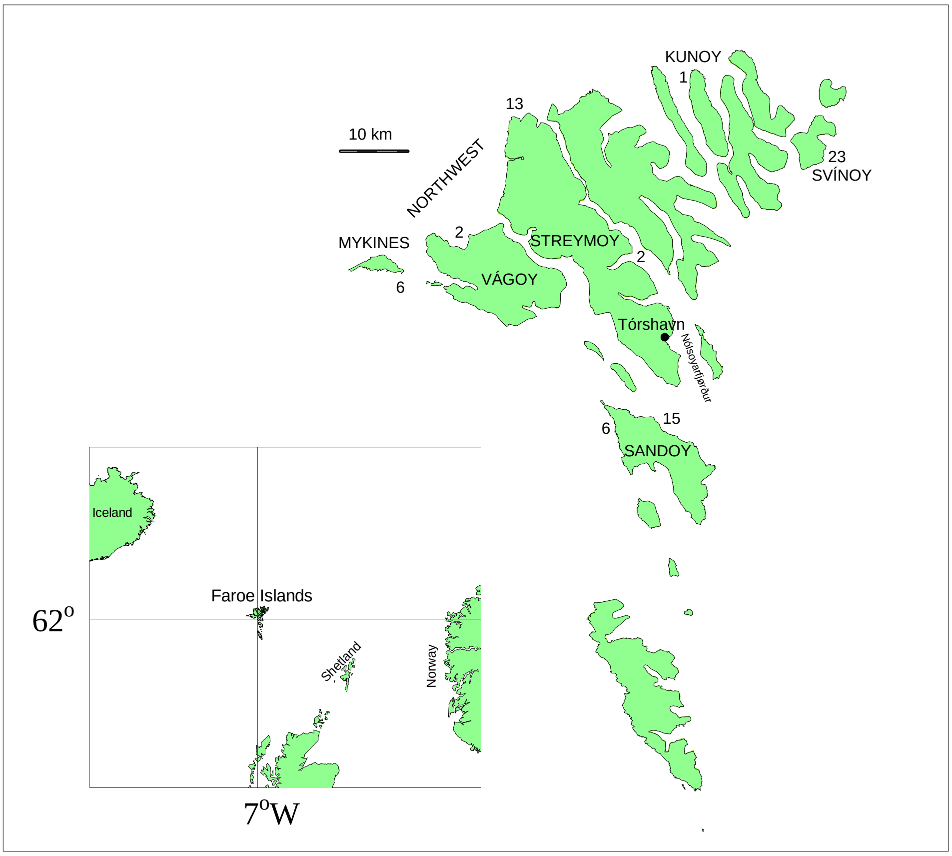
KUNOY
1
13
10 km
NORTHWEST
23
SVÍNOY
MYKINES
2
STREYMOY
2
6
VÁGOY
Tórshavn
Nólsoyarfjørður
6
15
SANDOY
Iceland
Faroe Islands
62°
Shetland
Norway
7°W

Figure 1

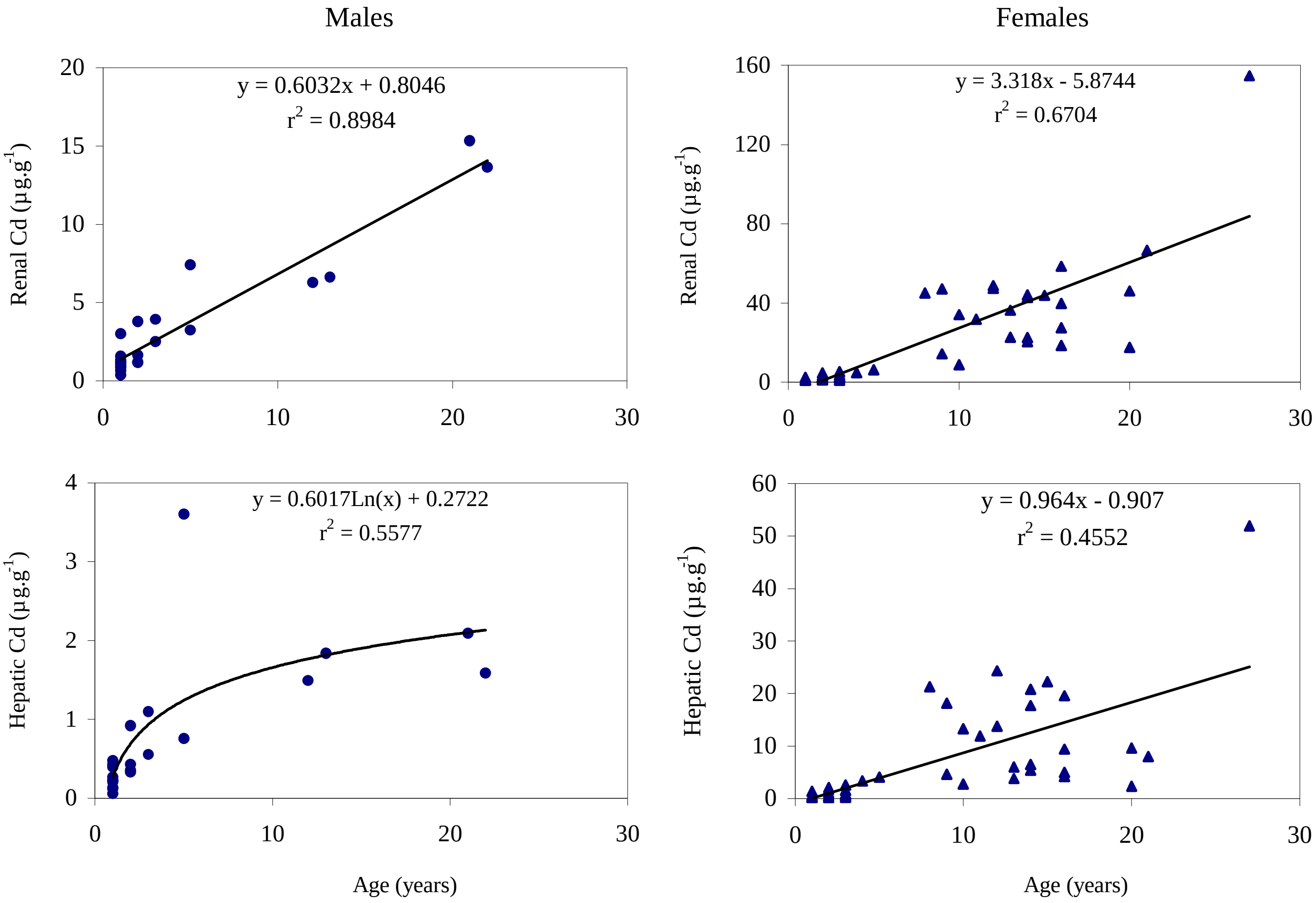
Males
Females
y = 0.6032x + 0.8046
r² = 0.8984
y = 3.318x - 5.8744
r² = 0.6704
Renal Cd (µg.g⁻¹)
Renal Cd (µg.g⁻¹)
20
15
10
5
0
160
120
80
40
0
0
10
20
30
0
10
20
30
y = 0.6017Ln(x) + 0.2722
r² = 0.5577
y = 0.964x - 0.907
r² = 0.4552
Hepatic Cd (µg.g⁻¹)
Hepatic Cd (µg.g⁻¹)
4
3
2
1
0
60
50
40
30
20
10
0
0
10
20
30
0
10
20
30
Age (years)
Age (years)

Figure 2

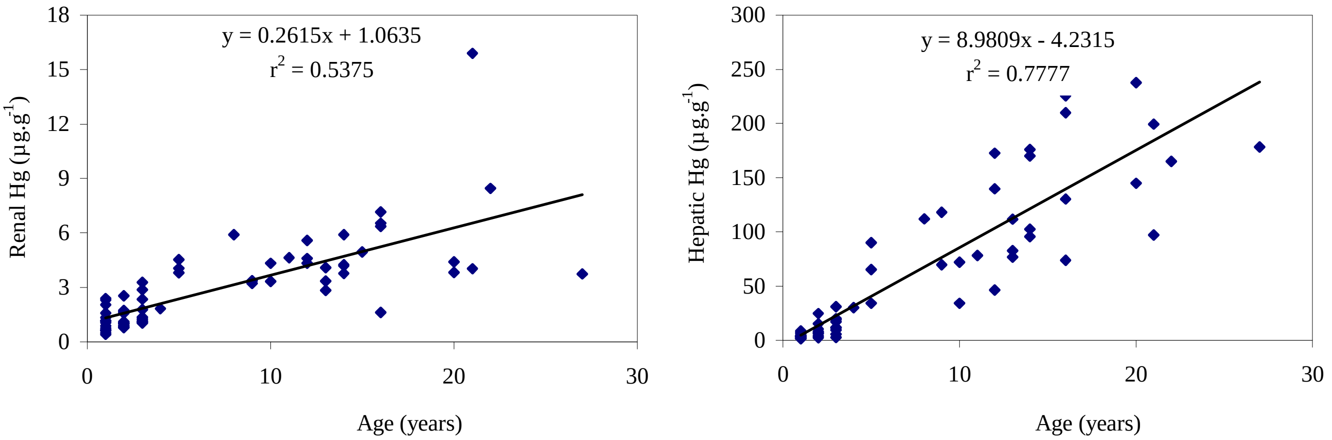
y = 0.2615x + 1.0635
$r^2 = 0.5375$
Renal Hg ($\mu g.g^{-1}$)
18
15
12
9
6
3
0
0
10
20
30
Age (years)
y = 8.9809x - 4.2315
$r^2 = 0.7777$
Hepatic Hg ($\mu g.g^{-1}$)
300
250
200
150
100
50
0
0
10
20
30
Age (years)

Figure 3

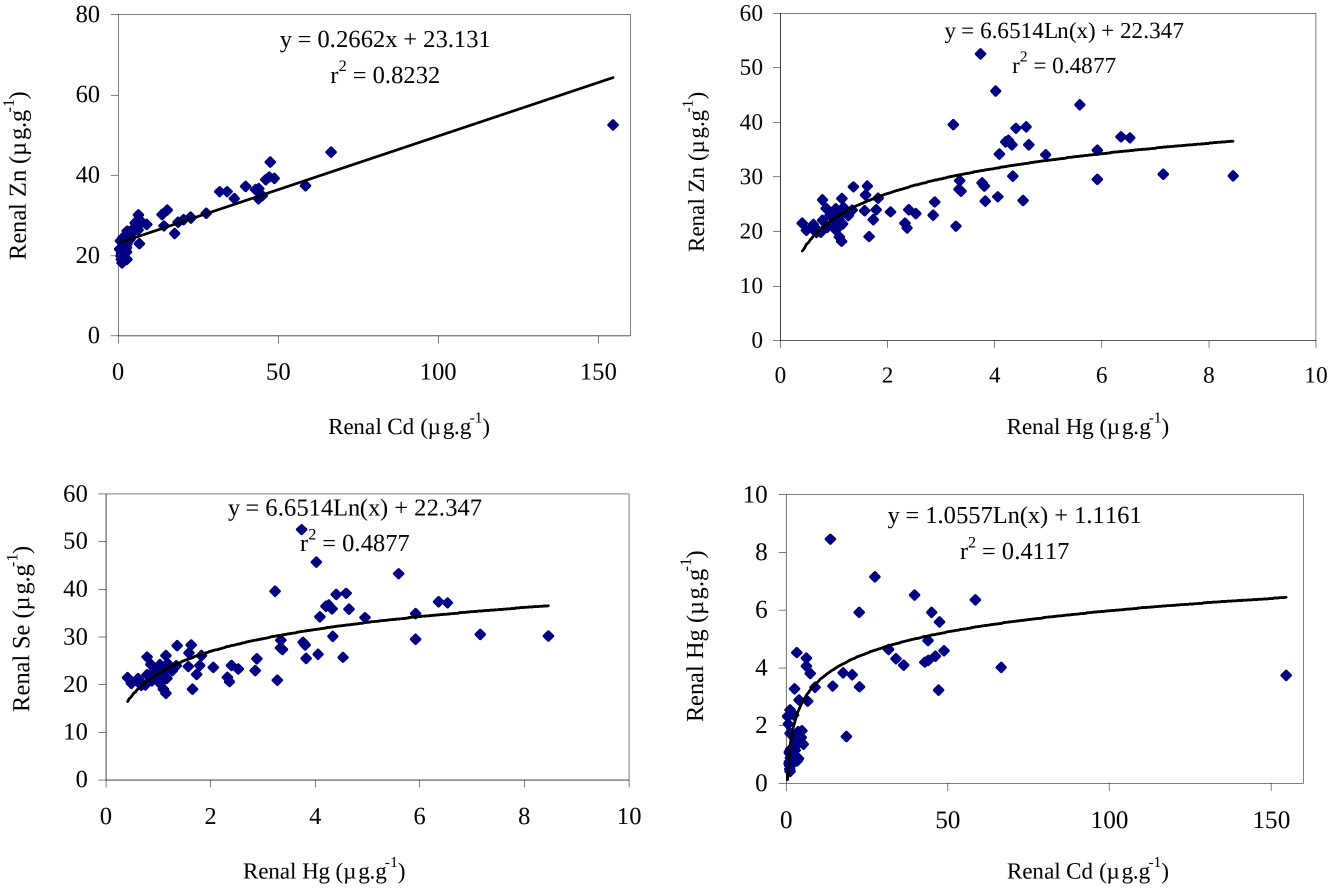
y = 0.2662x + 23.131
r² = 0.8232
Renal Zn (µg.g⁻¹)
0
20
40
60
80
0
50
100
150
Renal Cd (µg.g⁻¹)
y = 6.6514Ln(x) + 22.347
r² = 0.4877
Renal Zn (µg.g⁻¹)
0
10
20
30
40
50
60
0
2
4
6
8
10
Renal Hg (µg.g⁻¹)
y = 6.6514Ln(x) + 22.347
r² = 0.4877
Renal Se (µg.g⁻¹)
0
10
20
30
40
50
60
0
2
4
6
8
10
Renal Hg (µg.g⁻¹)
y = 1.0557Ln(x) + 1.1161
r² = 0.4117
Renal Hg (µg.g⁻¹)
0
2
4
6
8
10
0
50
100
150
Renal Cd (µg.g⁻¹)

Figure 4

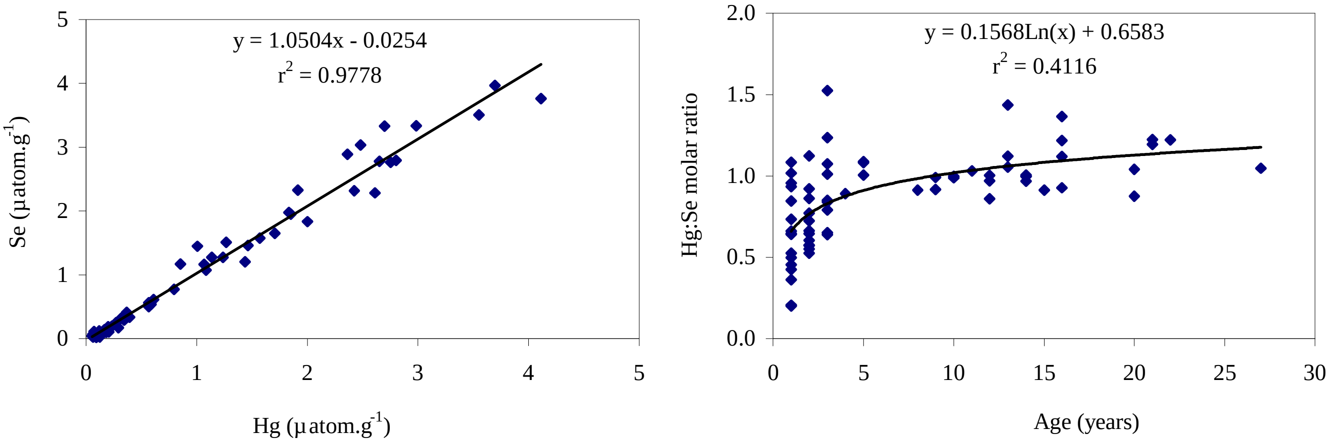
y = 1.0504x - 0.0254
r^2 = 0.9778
Se (µatom.g^{-1})
Hg (µatom.g^{-1})
0
1
2
3
4
5
y = 0.1568Ln(x) + 0.6583
r^2 = 0.4116
Hg:Se molar ratio
Age (years)
0.0
0.5
1.0
1.5
2.0
0
5
10
15
20
25
30

Figure 5